COMMUNICATION

A Partial Catalog of Proteins Secreted by Epidermal Keratinocytes in Culture

Anne B. Katz* and Lorne B. Taichman*†

Departments of *Oral Biology and Pathology and †Dermatology, SUNY at Stony Brook, Stony Brook, New York, U.S.A.

Proteins secreted by epidermal keratinocytes are known to engage in functions other than those directly associated with barrier formation. We have used a previously published culture model to collect proteins secreted by adult human epidermal keratinocytes. Electrophoresis and microsequencing allowed us to identify 20 proteins. The list of proteins includes those known to be produced by keratinocytes (β-2 microglobulin, βIG-H3, calgranulin A, cathepsin B and D, E-cadherin, gelatinase B, gelsolin, interstitial collagenase, laminin B2t, plasminogen activator inhibitor-1, protein 14–3-3ε, SCC antigen, stratifin, and translationally controlled tumor protein) as well as those not previously known to be secreted by keratinocytes (epididymis secretory protein, maspin, and anti-neoplastic urinary protein). In addition, two proteins were identified that are not known to be secreted (glutathione-S-transferase and heat shock protein 27/28 kDa). The varied nature of the proteins identified suggests that epidermal keratinocytes have physiologic functions that have yet to be identified. ***Key words: collagenase/extracellular protease/metabolic labeling. J Invest Dermatol 112:818–821, 1999***

Keratinocytes are the main cellular constituent of epidermis and generate a barrier by the formation of a stratified squamous keratinizing epithelium. In addition, keratinocytes secrete a variety of proteins that have diverse roles in epidermal physiology. For example, keratinocytes secrete collagen VII and laminin (Regauer *et al*, 1990; Marinkovich *et al*, 1993), which are utilized to form a basement membrane as well as proteinases and proteinase inhibitors for matrix remodeling (Clark, 1993). Keratinocytes also release a variety of cytokines, which have localized as well as systemic inflammatory and immunologic reactions (Schroder, 1992). And finally, keratinocytes secrete chemicals such as parathyroid hormone-related peptide (Wu *et al*, 1991), endothelin (Yohn *et al*, 1993) and the C3 complement component (Basset-Seguin *et al*, 1990). These nonbarrier functions of keratinocytes are noted by the production and release of various molecules to the extracellular space. The discovery of these secreted proteins usually comes about with some foreknowledge of their existence and/or function. For example, interleukin-1 was initially isolated from macrophages and observed to play a part in T cell activation. Because Langerhans cells share certain properties with macrophages, cultures of murine epidermal cells were tested and found to have interleukin-1 activity (Sauder *et al*, 1982). Detailed examination revealed that the source of interleukin-1 was not Langerhans cells but keratinocytes. The list of keratinocyte-secreted proteins is therefore restricted to those proteins for which some function or property is already known.

Celis and coworkers have compiled an extensive database of proteins released by primary human epidermal keratinocytes in suspension using two-dimensional electrophoresis and microsequencing (Rasmussen *et al*, 1992; Olsen *et al*, 1995). This method of identifying secreted protein did not rely upon prior knowledge of its presence or function. The viability of keratinocytes, however, is known to drop rapidly when the cells are placed in suspension (Rheinwald, 1979) and therefore, it is possible that some of the proteins were released as a result of cell lysis. Furthermore, it is not clear if changes in secretory activity were induced upon disaggregation of the tissue and disruption of the epidermal architecture.

In 1994 Katz and Taichman described a two-chamber keratinocyte culture model in which the keratinocytes formed a fully differentiated, stratified epithelium that was nourished from the undersurface and exposed to air (Katz and Taichman, 1994). In this model keratinocytes form a fully differentiated epithelium on a porous membrane in the upper chamber with fibroblasts and a serum-containing growth medium in the lower well. To analyze protein secreted by keratinocytes, the upper chamber is placed in a new well that lacks fibroblasts but contains a serum-free "collection medium". In this culture model, keratinocytes secrete 10–20 μg of protein per d per 10^6 cells of which only ≈3% is derived from lysed cells, as determined by the presence of minute amounts of involucrin in the medium. A small amount of serum contamination is present but this does not interfere with identification of proteins that are metabolically labeled. Metabolic labeling with ^{35}S-methionine and two-dimensional gel electrophoresis discloses about 70 proteins, many of which are likely to represent post-translational modifications. This method of collecting secreted proteins is also not dependent upon prior knowledge of function or structure and therefore provides an opportunity to survey secreted protein in an unbiased way. In this report we have collected ^{35}S-labeled protein synthesized and secreted by adult epidermal keratinocytes and using electrophoresis and microsequencing, managed to identify 20 proteins, some of which are quite unexpected.

Human, adult epidermal keratinocytes were maintained with the aid of γ-irradiated 3T3 cells (Rheinwald and Green, 1975) in a

Manuscript received June 17, 1998; revised October 19, 1998; accepted for publication January 20, 1999.

Reprint requests to: Lorne Taichman, SUNY at Stony Brook, Westchester Hall (Room 201), Stony Brook, NY 11794-8702, U.S.A.

Table I. Keratinocyte-secreted proteins[a]

	Protein	FW	Sequence identified[d]	Amino acid position in protein	Selected references
1	Gelsolin	85 697	ATASRGASQAG	28–38[f]	Kwiatkowski *et al*, 1988 Kubler and Watt, 1993 Olsen *et al*, 1995
2	Laminin B2t	130 871	DENPDIEXAD	435–444[f]	Sugiyama *et al*, 1995
3	Gelatinase B (MMP9)	78 384	APRQRQXXL	20–28[f]	Salo *et al*, 1994 Birkedal-Hansen *et al*, 1993
4	E-cadherin	97 456	DXVIPPIXXLENK	155–168[f]	Kemler, 1993 Bussemakers *et al*, 1993
5	βIG-H3	74 680	ASLPAEVLDSLVSNV[e]	149–163[e]	LeBaron *et al*, 1995
6	Interstitial collagenase (MMP1)	54 007	FPATLETQEQDVDLV	20–34[e]	SaarialhoKere *et al*, 1995 Petersen *et al*, 1990
7	Plasminogen activator inhibitor-1	45 060	VHHPXXYVAXLA	24–35[f]	Staiano-Coico *et al*, 1996
8	Cathepsin B	37 807	RXRPSFHPV	18–26[f]	Kawada *et al*, 1997 Keppler *et al*, 1996
9	Maspin[b]	42 138	TNPSTMANAKV[e]	259–269[f]	Zou *et al*, 1994
10	Cathepsin D	44 552	LVRIPLHKFTXXR	21–33 (5)	Kawada *et al*, 1997 Kawada *et al*, 1996
11	SCC antigen (leupin)	44 534	AATYHVDRSG	70–79	Olsen *et al*, 1995 Suminami *et al*, 1991 Schneider *et al*, 1995
12	Protein 14–3-3ε	29 174	RX EQXEENXGGED[e]	61–77	Aitken, 1995
13	Stratifin (14–3-3σ)	27 774	RVLSSIEQKSNEEGSEEKGP[e]	60–79	Leffers *et al*, 1993 Hermeking *et al*, 1997
14	Heat shock protein 27/28 KD[c]	22 327	RVSLDVNHFAPDELT[e]	96–110	Edwards *et al*, 1991 Kindas-Mugge and Trautinger, 1994
15	Glutathione-S-transferase[c]	23 356	PPYTVVYFPVRGRXA	2–16	Shimizu *et al*, 1995 Blacker *et al*, 1991 Vessey *et al*, 1995
16	Translationally controlled tumor protein	19 595	MIIYRDLISHDEMFS	1–15	Gross *et al*, 1989 Bohm *et al*, 1991 Sanchez *et al*, 1997
17	β-2 microglobulin	13 714	IQRTPKIQVYSRHPA	21–35[e]	Symington, 1989 Harrist *et al*, 1983
18	Calgranulin A	10 834	MLTELEKALNSIXDV	1–15	Saintigny *et al*, 1992 Madsen *et al*, 1992
19	Epididymis secretory protein[b]	16 570	EPVQFKDXGXVD	20–31[f]	Kirchhoff *et al*, 1991 Kirchhoff *et al*, 1991
20	Anti-neoplastic urinary protein	1674	LKXYTXKEPMTSA	2–14	Ridge and Sloane, 1996

[a]Proteins secreted by epidermal keratinocytes in culture were fractionated by two-dimensional gel electrophoresis and microsequencing was performed on a number of distinct spots. Identification of the full length protein was made possible by comparison with known sequences in protein data banks.
[b]Protein not previously identified in keratinocytes.
[c]Protein not known to be secreted.
[d]Amino acids identified by microsequencing are denoted by a single letter according to standard IUB/IUPAC amino acid codes. An 'X' denotes an amino acid that could not be identified by microsequencing.
[e]Microsequencing was performed from an *N*-chlorosuccinimide fragment.
[f]The position of the amino acids in the precursor protein is noted.

growth medium containing a 3:1 mix of Dulbecco's modified Eagle medium/F12, 5% fetal calf serum, adenine, insulin, epidermal growth factor, cholera enterotoxin, HEPES, penicillin, and streptomycin in amounts described elsewhere (Wu *et al*, 1982). At pass three to seven keratinocytes (2×10^6 cells) were seeded into 24 mm Transwell Nucleopore polycarbonate tissue inserts (Costar, Cambridge, MA) with 3×10^6 γ-irradiated 3T3 in the lower chamber as described (Katz and Taichman, 1994). In some experiments 100 mm tissue inserts were seeded with 2.5×10^7 keratinocytes with 1.5×10^7 γ-irradiated 3T3 in the lower chamber. On day 3, medium was removed from the upper chamber and thereafter keratinocytes were nourished from the undersurface from medium in the lower chamber. On days 10–15, secreted protein was recovered in collection medium consisting of Dulbecco's modified Eagle medium/F12, hydrocortisone, adenine, insulin, and HEPES. To remove residual serum protein, tissue inserts were put through two cycles of two rinses with phosphate-buffered saline (2 ml per rinse in the lower well) plus one incubation in collection medium (1.3 ml for 1 h in the lower well). Secreted protein was recovered in collection medium over a 16 h period and stored at –80°C with phenylmethylsulfonyl fluoride (100 μg per ml). Repeated collections of secreted protein from the same insert could be performed by allowing an 8 h period in growth medium between collections. In this way secreted protein could be harvested up to five times from a single insert. Protein in the collection medium was used only if the lactic acid dehydrogenase was less than 100 IU. The amount of secreted protein in the medium ranged from 15 to 25 μg per ml. Metabolic labeling of secreted protein was achieved with 24 mm inserts by adding 143°μCi S^{35}-met (1175°μCi per mmol, New England Nuclear, Boston, MA) to medium in the lower chamber for a period of 6 h.

Nonlabeled secreted protein was precipitated with 2 volumes of acetone at –20°C for 1 h and collected by centrifugation at $7700 \times g$ for 40 min at 4°C. Proteins were resuspended and dialyzed against water and then lyophilized (SpeedVac, Savant Instruments, Farmingdale, NY). Metabolically labeled secreted protein was put through three cycles of acetone precipitation and resuspended in water, and used without dialysis or lyophilization. Electrophoresis in the first dimension was by isoelectric focusing (Dunbar *et al*, 1990). For each gel, 800–1000 μg of nonlabeled protein and 1×10^6 cpm of S^{35}-labeled protein were combined, dissolved in urea buffer and electrophoresed for 19,400 Vh in a tube

2.5 × 140 mm. The isoelectric focusing gel was suspended in equilibration buffer and either frozen (–80°C) for use within 10 d or run directly in the second dimension (Sambrook *et al*, 1989). The sodium dodecyl sulfate/polyacrylamide gel electrophoresis gel was transferred to Immobilon PSQ (Millipore, Bedford, MA) using Tris-glycine buffer (39 mM glycine, 48 mM Tris base, 0.037% sodium dodecyl sulfate, 20% methanol) overnight at 30 V. The Immobilon was stained with 0.25% Coomassie Blue in a mixture of methanol/water/acetic acid (45:45:10), destained, and exposed to X-ray film. Spots which were both radioactive and Coomassie-Blue-positive were cut out and sequenced by the Center for Analysis and Synthesis of Macromolecules (SUNY at Stony Brook) using a Perkin-Elmer (Applied Biosystem Division, Foster City, CA) Model 475A protein sequencer and standard cycles. When the protein to be sequenced was blocked at its N terminal, the protein was treated with *N*-chlorosuccinimide (Lischwe and Ochs, 1982) and run on a 15% resolving gel. Internal fragments were used for microsequencing. To obtain sufficient material, especially for low molecular weight protein, 26 mg of secreted protein was prefractionated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis tube gels (18 × 5 cm). The gels were cut into five 1 cm sections and similar sections combined and electroluted into a dialysis bag. The eluted protein was then dialyzed against water and concentrated by lyophilization. Prefractionated proteins from one section were combined with S^{35}-labeled protein, focused on an isoelectric focusing gel and run in the second dimension on a 6%, 10%, or 15% gel, depending on the size range of the protein. The amino acid sequence obtained was compared with sequences in Brookhaven Protein Data Bank, GeneBank, GeneBank updates, EMBL Data Library and EMBL Data Library updates using the BLAST E-mail server at NIH (BLAST@ncbi.nlm.nih.gov).

For a protein to be analyzed, it must be present in amounts sufficient to form a visible spot on a Coomassie-stained blot. Generally, this corresponds to ≈5 μg of protein. Protein secreted in amounts below this will not be identified. For example, we know that keratinocytes secrete apolipoprotein E at a rate of 0.97 ng per h per 10^6 cells (Gordon *et al*, 1989). Apolipoprotein E was not identified in our analysis. In spite of our efforts to remove serum albumin from the cultures, some was always present in the medium and this interfered with analysis in that region of the gel. It should also be noted that only five of the 19 proteins identified were blocked at the amino terminus and sequencing was performed on an internal *N*-chlorosuccinimide fragment. As the amino terminus of a secreted protein encodes secretory properties and is usually removed during membrane translocation, the low number of blocked proteins is not unexpected.

Proteins identified in this way are listed in **Table I** and their position on two-dimensional gel electrophoresis is shown in **Fig 1**. Many of the proteins have been identified previously by Celis and coworkers (Rasmussen *et al*, 1992; Olsen *et al*, 1995). Most of the proteins identified are known or thought to be involved in matrix remodeling, either as a proteinase or as a proteinase inhibitor (cathepsin B and D, epididymis secretory protein, gelatinase B, interstitial collagenase, maspin, plasminogen activator inhibitor-1, and SCC antigen). One protein (laminin B2t) is a structural component of the basement membrane and another (β-2 microglobulin) is a component of the major histocompatibility complex antigen. One protein (βIG-H3) promotes adhesion and spreading of fibroblasts, another (calgranulin) has similarity to fatty acid binding proteins and one (gelsolin) has actin binding properties. Anti-neoplastic urinary protein is a poorly characterized protein that has not been previously identified in keratinocytes and translationally controlled tumor protein has no known function. Two proteins (protein 14–3-3ε and stratifin) are not known to be secreted but have been recovered in the keratinocyte culture medium (Celis *et al*, 1994). The significance of this is unclear. Two proteins (glutathione-S-transferase and heat shock protein 27/28 KD) are not known to be secreted and the explanation for their presence in the medium is unclear. It is unlikely that these two proteins are contaminants from cell lysis because lactic acid dehydrogenase levels were <100 IU in all batches of medium used for protein isolation. In addition, we know from reconstruction experiments that ≈3% of the metabolically labeled protein recovered in the medium of these lifted cultures can be accounted for by cell lysis (Katz and Taichman, 1994). It is possible that the amino acid sequence identified by microsequencing is shared by or is similar to sequences present in other secreted proteins. Secreted proteins are likely to have functions that involve other cells, either in close proximity or at some distance. The secreted proteins identified in this study may stimulate further research into other, as yet to be identified, functions of epidermis.

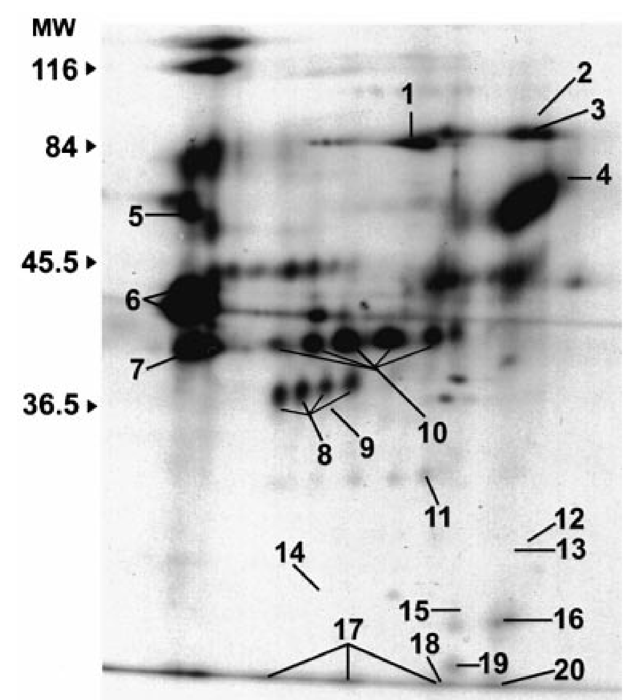

Figure 1. Identification of keratinocyte-secreted protein. Secreted protein metabolically labeled with ^{35}S-met was analyzed by two-dimensional gel electrophoresis. The two-dimensional gel electrophoretic pattern shown here has been described previously (Katz and Taichman, 1994). Peptide mapping indicates that the proteins labeled as nos 8 and 10 are post-translational modifications. The protein spot actually sequenced is identified by the straight line. Further information on each protein is provided in **Table I**.

This research was funded by a grant from the Center International de Recherches Dermatologiques GALDERMA (Sophia Antipolis, France). We thank Dr. Uwe Reichert (CIRD GALDERMA) for his assistance and support. We thank Thomas Fischer (Center for Analysis and Synthesis of Macromolecules, SUNY Stony Brook) for his help in identifying secreted protein. We also thank members of our laboratory for their suggestions and helpful comments.

REFERENCES

Aitken A: 1433 proteins on the MAP. *Trends Biochem Sci* 20:95–97, 1995

Basset-Seguin N, Caughman SW, Yancey KB: A431 cells and human keratinocytes synthesize and secrete the third component of complement. *J Invest Dermatol* 95:621–625, 1990

Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA: Matrix metalloproteinases: a review. [Review]. *Crit Rev Oral Biol Med* 4:197–250, 1993

Blacker KL, Olson E, Vessey DA, Boyer TD: Characterization of glutathione S-transferase in cultured human keratinocytes. *J Invest Dermatol* 97:442–446, 1991

Bohm H, Gross B, Gaestel M, Bommer UA, Ryffel G, Bielka H: The 5′untranslated region of p23 mRNA from the Ehrlich ascites tumor is involved in translation control of the growth related protein p23. *Biomed Biochim Acta* 50:1193–1203, 1991

Bussemakers MJ, van Bokhoven A, Mees SG, Kemler R, Schalken JA: Molecular cloning and characterization of the human E-cadherin cDNA. *Mol Biol Rep* 17:123–128, 1993

Celis JE, Rasmussen HH, Olsen E, *et al*: The human keratinocyte two-dimensional protein database (update 1994): towards an integrated approach to the study of cell proliferation, differentiation and skin diseases. *Electrophoresis* 15:1349–1458, 1994

Clark RA: Biology of dermal wound repair. *Dermatol Clinics* 11:647–666, 1993

Dunbar BS, Kimura H, Timmons TM: Protein analysis using high-resolution two-dimensional polyacrylamide gel electrophoresis. *Methods Enzymol* 182:441–459, 1990

Edwards MJ, Marks R, Dykes PJ, Merrett VR, Morgan HE, O'Donovan MR: Heat shock proteins in cultured human keratinocytes and fibroblasts. *J Invest Dermatol* 96:392–396, 1991

Gordon DA, Fenjves ES, Williams DL, Taichman LB: Synthesis and secretion of apolipoprotein E by cultured human keratinocytes. *J Invest Dermatol* 92:96–99, 1989

Gross B, Gaestel M, Bohm H, Bielka H: cDNA sequence coding for a translationally controlled human tumor protein. *Nucleic Acids Res* 17:8367, 1989

Harrist TJ, Ruiter DJ, Mihm MC Jr, Bhan AK: Distribution of major histocompatibility antigens in normal skin. *Br J Dermatol* 109:623–633, 1983

Hermeking H, Lengauer C, Polyak K, *et al*: 1433_ is a p53regulated inhibitor of G2/M progression. *Mol Cell* 1:3–11, 1997

Katz AB, Taichman LB: Epidermis as a secretory tissue: an *in vitro* tissue model to study keratinocyte secretion. *J Invest Dermatol* 102:55–60, 1994

Kawada A, Hara K, Kominami E, Kobayashi T, Hiruma M, Ishibashi A: Cathepsin B and D expression in squamous cell carcinoma. *Br J Dermatol* 135:905–910, 1996

Kawada A, Hara K, Kominami E, Hiruma M, Noguchi H, Ishibashi A: Processing of cathepsins L, B and D in psoriatic epidermis. *Arch Dermatol Res* 289:87–93, 1997

Kemler R: From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet* 9:317–321, 1993

Keppler D, Sloane BF: Cathepsin B: multiple enzyme forms from a single gene and their relation to cancer. *Enzyme Protein* 49:94–105, 1996

Kindas-Mugge I, Trautinger F: Increased expression of the M(r) 27,000 heat shock protein (hsp27) in in vitro differentiated normal human keratinocytes. *Cell Growth Differ* 5:777–781, 1994

Kirchhoff C, Habben I, Ivell R, Krull N: A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. *Biol Reprod* 45:350–357, 1991a

Kubler MD, Watt FM: Changes in the distribution of actin-associated proteins during epidermal wound healing. *J Invest Dermatol* 100:785–789, 1993

Kwiatkowski DJ, Mehl R, Yin HL: Genomic organization and biosynthesis of secreted and cytoplasmic forms of gelsolin. *J Cell Biol* 106:375–384, 1988

LeBaron RG, Bezverkov KI, Zimber MP, Pavelec R, Skonier J, Purchio AF: Beta IGH3, a novel secretory protein inducible by transforming growth factor-beta, is present in normal skin and promotes the adhesion and spreading of dermal fibroblasts in vitro. *J Invest Dermatol* 104:844–849, 1995

Leffers H, Madsen P, Rasmussen HH, *et al*: Molecular cloning and expression of the transformation sensitive epithelial marker stratifin. A member of a protein family that has been involved in the protein kinase C signalling pathway. *J Mol Biol* 231:982–998, 1993

Lischwe MA, Ochs D: A new method for partial peptide mapping using N-chlorosuccinimide/urea and peptide silver staining in sodium dodecyl sulfate polyacrylamide gels. *Anal Biochem* 127:453–457, 1982

Madsen P, Rasmussen HH, Leffers H, Honore B, Celis JE: Molecular cloning and expression of a novel keratinocyte protein (psoriasis-associated fatty acid-binding protein [PAFABP]) that is highly upregulated in psoriatic skin and that shares similarity to fatty acid-binding proteins. *J Invest Dermatol* 99:299–305, 1992

Marinkovich MP, Keene DR, Clytie SR, Burgeson RE: Cellular origin of the dermal-epidermal basement membrane. *Dev Dynamics* 197:255–267, 1993

Olsen E, Rasmussen HH, Celis JE: Identification of proteins that are abnormally regulated in differentiated cultured human keratinocytes. *Electrophoresis* 16:2241–2248, 1995

Petersen MJ, Woodley DT, Stricklin GP, O'Keefe EJ: Enhanced synthesis of collagenase by human keratinocytes cultured on type I or type IV collagen. *J Invest Dermatol* 94:341–346, 1990

Rasmussen HH, van Damme J, Puype M, Gesser B, Celis JE, Vandekerckhove J: Microsequences of 145 proteins recorded in the two-dimensional gel protein database of normal human epidermal keratinocytes. *Electrophoresis* 13:960–969, 1992

Regauer S, Seiler GR, Barrandon Y, Easley KW, Compton CC: Epithelial origin of cutaneous anchoring fibrils. *J Cell Biol* 111:2109–2115, 1990

Rheinwald JG: The role of terminal differentiation in the finite culture lifetime of the human epidermal keratinocyte. *Int Rev Cytol Suppl* 10:25–33, 1979

Rheinwald JG, Green H: Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinocyte colonies from single cells. *Cell* 6:331–344, 1975

Ridge RJ, Sloane NH: Partial N-terminal amino acid sequence of the antineoplastic urinary protein (ANUP) and the antitumour effect of the N-terminal nonapeptide of the unique cytokine present in human granulocytes. *Cytokine* 8:1–5, 1996

SaarialhoKere UK, Vaalamo M, Airola K, Niemi KM, Oikarinen AI, Parks WC: Interstitial collagenase is expressed by keratinocytes that are actively involved in reepithelialization in blistering skin disease. *J Invest Dermatol* 104:982–988, 1995

Saintigny G, Schmidt R, Shroot B, Juhlin L, Reichert U, Michel S: Differential expression of calgranulin A and B in various epithelial cell lines and reconstructed epidermis. *J Invest Dermatol* 99:639–644, 1992

Salo T, Makela M, Kylmaniemi M, AutioHarmainen H, Larjava H: Expression of matrix metalloproteinase-2 and −9 during early human wound healing. *Lab Invest* 70:176–182, 1994

Sambrook J, Fritsch EF, Maniatis T: Molecular cloning. *A Laboratory Manual*, 2nd edn. Cold Spring Harbor: Cold Spring Harbor Laboratory, 1989

Sanchez JC, Schaller D, Ravier F, *et al*: Translationally controlled tumor protein: a protein identified in several nontumoral cells including erythrocytes. *Electrophoresis* 18:150–155, 1997

Sauder DN, Carter CS, Katz SI, Oppenheim JJ: Epidermal cell production of thymocyte activating factor (ETAF). *J Invest Dermatol* 79:34–39, 1982

Schneider SS, Schick C, Fish KE, *et al*: A serine proteinase inhibitor locus at 18q21.3 contains a tandem duplication of the human squamous cell carcinoma antigen gene. *Proc Natl Acad Sci USA* 92:3147–3151, 1995

Schroder JM: Chemotactic cytokines in the epidermis. *Exp Dermatol* 1:12–19, 1992

Shimizu K, Toriyama F, Yoshida H: The expression of placental-type glutathione S-transferase (GSTpi) in human cutaneous squamous cell carcinoma and normal human skin. *Virch Arch* 425:589–592, 1995

Staiano-Coico L, Carano K, Allan VM, *et al*: PAI1 gene expression is growth state-regulated in cultured human epidermal keratinocytes during progression to confluence and postwounding. *Exp Cell Res* 227:123–134, 1996

Sugiyama S, Utani A, Yamada S, Kozak CA, Yamada Y: Cloning and expression of the mouse laminin gamma 2 (B2t) chain, a subunit of epithelial cell laminin. *Eur J Biochem* 228:120–128, 1995

Suminami Y, Kishi F, Sekiguchi K, Kato H: Squamous cell carcinoma antigen is a new member of the serine protease inhibitors. *Biochem Biophys Res Commun* 181:51–58, 1991

Symington FW: Lymphotoxin, tumor necrosis factor, and gamma interferon are cytostatic for normal human keratinocytes. *J Invest Dermatol* 92:798–805, 1989

Vessey DA, Lee KH, Boyer TD: Differentiation-induced enhancement of the ability of cultured human keratinocytes to suppress oxidative stress. *J Invest Dermatol* 104:355–358, 1995

Wu YJ, Parker LM, Binder NE, *et al*: The mesothelial keratins: a new family of cytoskeletal proteins identified in cultured mesothelial cells nonkeratinizing epithelia. *Cell* 31:693–703, 1982

Wu TL, Soifer NE, Burtis WJ, Milstone LM, Stewart AF: Glycosylation of parathyroid hormone-related peptide secreted by human epidermal keratinocytes. *J Clin Endocrinol Metab* 73:1002–1007, 1991

Yohn JJ, Morelli JG, Walchak SJ, Rundell KB, Norris DA, Zamora MR: Cultured human keratinocytes synthesize and secrete endothelin1. *J Invest Dermatol* 100:23–26, 1993

Zou Z, Anisowicz A, Hendrix MJ, *et al*: Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells [see comments]. *Science* 263:526–529, 1994